REMARKS/ARGUMENTS

Claims 1, 3, 13-16, 18, 24, 25, 30-35, and 45-46 are active. Claims 4-10, 17, 19, 20, 26-29, and 36-44 have been withdrawn from consideration. Claim 1 has been revised for clarity and to point out specific aspects of the invention. Support for this amendment is found in the specification in the paragraph bridging pages 2-3 and in original Claims 1, 12 and 23. The dependent claims have been amended consistently with Claim 1. Accordingly, the Applicants do not believe that any new matter has been introduced.

The Applicants thank Examiner Dunston for the courtesies extended during the interview of June 9, 2005. It was indicated that limiting the scope for the claims to the elected species (MRP1, spacer, Kir6.2) would overcome the prior art rejections. To address the enablement rejection, the Applicants were encouraged to point out the functionality of the elected species, e.g., that the claimed hybrid protein functions as an electrical sensor. The Applicants provide herewith a Declaration regarding functionality of the elected species of hybrid protein (MRP1-Kir6.2, e.g., SEQ ID NO: 1).

Claim 1 has now been revised to refer to an ABC transporter membrane protein, a spacer and a potassium ion channel protein of the Kir family, and to indicate that these elements are functionally couple to produce an electrical signal. Favorable consideration of this amendment and allowance of this application is now respectfully requested.

Election/Restriction

The Applicants previously elected Group I (hybrid protein), and elected a hybrid protein species comprising (a) a spacer, (b) MRP-1 and (c) Kir6.2. This requirement has been made FINAL. The Applicants understand that additional species falling within an elected generic claim, such as Claim 1, will be searched upon an indication of allowability for the generic claim as it reads on the presently elected species. They also respectfully request

rejoinder of nonelected claims which depend from, or which otherwise include all the limitations of an allowable elected claim.

Sequence Compliance

The specification was objected to as referring to FKYENE, but as not identifying this sequence using a SEQ ID NO. This objection is moot in view of the revision of page 5 of the specification to identify this sequence using SEQ ID NO: 25.

Objection—Abstract

The Abstract was objected to as containing legal phraseology. This objection is moot in view of the attached revised Abstract.

Objections-Claims

Claims 25, 45 and 46 were objected to for various informalities. The Applicants respectfully request that Claim 25 be examined as it reads on other nonelected species, upon an indication of allowability for this claim as it encompasses the elected species. Claims 45 and 46 have been revised as suggested by the Examiner. To avoid the possibility that the words "contaminant" and "pollutant" might later be construed differently (e.g., biological contaminant, chemical pollutant), for clarity, the terms "contaminant" and "pollutant" have both been retained, however, the Applicants have revised this term to refer to these words alternatively by interposing the word "or" between them.

Rejections—35 U.S.C. §112, first paragraph

Claims 1-3, 11-13, 15, 21-24, 34, 35, 45 and 46 were rejected under 35 U.S.C. 112, first paragraph, as lacking adequate written description and enablement. The Applicants

traverse this rejection because the specification adequately describes the terms in the claims, such as "membrane protein" and "ion channel". These terms would be readily understood by one with skill in the art and, while broad, are adequately described. Nevertheless, Claim 1 has been revised to further characterize both the type of membrane protein (ABC transporter) and ion channel (Kir family potassium channel).

Moreover, to the extent that examination has been limited to the elected species (MRP1, spacer, Kir6.2) that Applicants submit that each element has been more than adequately described and enabled, since this species is actually exemplified (SEQ ID NO: 1) in the specification. Accordingly, the Applicants respectfully request that this rejection now be withdrawn.

Operability of the elected species (MRP1, spacer, Kir6.2)

The Examiner's concern expressed in the interview and the Official Action was that ligand binding to the membrane component (MRP1) would not transduce a signal to the Kir6.2 ion channel and thus the hybrid protein would not function as an electrical sensor. The Applicants attach herewith the Declaration of Michel Vivaudou which addresses this issue and further shows signal transduction in the hybrid protein when contacted with a suitable ligand.

Briefly, Fig. 2 of the Declaration shows the effects of a known substrate for MRP1 (glibenclamide) on the channel activity of the fusion protein MRP1-Kir6.2. The ion channels in the hybrid proteins are significantly inhibited by the known MRP1 substrate.

Fig. 1 of the Declaration shows that ion channel activity in a MDR1-Kir6.2 fusion protein is also significantly inhibited by known MDR1 substrates.

These results show that signal transduction is occurring since substrates which bind to MRP1 or MRP1 inhibit Kir6.2 ion channel activity.

While the Declaration points out that nucleotide binding to MRP1 does not induce a change in Kir6.2 activity, the aim of the invention is not to record the binding of nucleotides which target cytosolic domains of ABC transporters and thus would be of little interest in screening procedures. On the other hand, the results shown in Fig. 2 clearly show that substrate binding to the relevant portions of the MRP1-Kir6.2 protein modulates ion channel activity.

As discussed above, the claims have now been directed to hybrid proteins comprising ABC transporters. Indeed, it must be pointed out that all the ABC transporters solve the instant problem, i.e., being effectively electrical sensors of said ABC transporter activity, for studying said ABC transporter and for detecting and screening molecules of interest. Thus, the inventors have found, unexpectedly, that there exists a functional coupling between ABC transporter and Kir6.2 (ion channel receptor): in the ion-channel-receptor/ABC transporter hybrid protein, the receptor/transporter occupancy by ligand/substrate is transferred to the ion channel (Kir6.2) and transduced into an electrical signal that is detected by standard electrophysiological techniques (patch clamp technique or two-microelectrode voltage clamp, for instance).

In the herewith attached Declaration, one of the inventors, M.M. VIVAUDOU confirms that different ABC transporters coupled to Kir 6.2 or in some cases to a slightly modified Kir6.2 protein effectively signal transduce and thus show the operability of the hybrid proteins of the invention as sensors.

In the attached Declaration, M. M. VIVAUDOU shows that figure 1 relating to MDR1 shows evidence that MDR1-Kir6.2 hybrid protein is properly folded, trafficked and inserted into the membrane, such that a functional channel is formed. Such hybrid protein is well expressed and is modulated by several substrates of MDR1.

Considering figure 1 and the data provided in the specification, MRP1, MDR1 and YCF1 coupled to Kir6.2 or Kir6,2ΔC36 are functional channels and are able to be used as electrical sensors.

As regards the objection of the Examiner, concerning the hybrid protein MRP1-Kir6.2 (page 11 of the Office Action), the following may be pointed out:

It is true that **nucleotide** binding to MRP1 does not induce a change in Kir6.2 channel activity. However, the aim of the instant invention is not to record the binding of nucleotides which target cytosolic domains of ABC transporters and are of little interest for screening, but the binding of substrates or modulators which are known to target the transmembrane domains of MRP1.

As shown, in the herewith attached figure 2, the inventors show that binding of substrate to MRP1 can indeed measurably modulate channel activity.

This is also clearly specified on page 2, lines 26-30 of the specification.

In this context, Baukrowitz et al. document is not pertinent.

As regards the objection of the Examiner, concerning the predictability of the invention over the state of the art, the following may be pointed out:

Enablement or operability of an invention does not depend on whether the inventors have completely characterized the mechanism of action. Many patents have been granted, especially in the health care field, without knowing exactly the mechanism of action underlying the found activity. This applies for instance to sulfonylurea, which was used for a long time for treating diabetes mellitus; the mechanism of action having been discovered only in the 1990's.

The inventors had full success in constructing and expressing fusion proteins based on numerous ABC transporters (MRP1, YCF1, MDR1, etc). The molecular biological and

biochemical techniques involved are straightforward for the skilled artisan. The obtained results are exposed in herewith in Figs. 1 and 2 of the attached Declaration.

Figure 1 represents the effects of MDR1 substrates on channel activity of fusion protein MDR1-Kir6.2 measured in *Xenopus* oocyte excised patches (see method, page 17 of the instant specification). Comparison with the construct MDR1 (TMD0SUR2A) +Kir6.2 where the N-terminal domain TMD0 of SUR2A, which have been identified as the domain interacting with Kir6.2, is fused at the N-terminal end of MDR1. The latter construct assembles spontaneously with Kir6.2. Both constructs are significantly inhibited by the tested compounds.

Figure 2 shows the effect of glibenclamide, a known substrate of MRP1 substrates on channel activity of fusion protein MRP1-Kir6.2. The trace shown is a patch clamp record measured in *Xenopus* oocyte in the excised inside-out configuration. Hybrid channels are significantly inhibited by the tested compound.

Even though the inventors agree with the fact that the intimate details of the interactions of SUR and Kir6.2 are not known and will not be known as long as high-resolution structures or adequate molecular models are not available, this does not constitute a bar to the instant invention, knowing that even without structures, details of the interaction SUR/Kir6.2 have emerged with regions involved being identified (see figure 1 annexed).

Indeed, contrary to the assertions of the Official Action and as shown in the herewith attached documents relating to K^+ channels (Bienengraeber, et al., FASEB J., 2000, 14, 1943-1952; Giblin, et al., J. Biol. Chem., 2002, 277, 16,13717-13723; Heinemann, Encyclopedia of Life Sciences, 2001), there is no need to have the knowledge of the mechanism of action if the product is characterized and if tests exist which verify the properties of said product.

Undue Experimentation. The Applicants traverse this basis of rejection since the

invention as now claimed is clearly directed to specific, well-known classes of membrane and ion channel proteins. The following elements are well-known.

-nucleic acid sequences encoding an ABC transporter: all the sequences are available in the data bases; furthermore there exist many publications giving the structure of ABC transporters. ABC transporters are known to contain two ATP binding domains and 12 transmembrane domains which are believed to form a channel-like structure are essential for the transport function of ABC proteins.

-nucleic acid sequences encoding Kir6.2 or a derivative thereof; Kir6.2 have been described, for instance by Inagaki et al., Science, 1995, 270, 1166-1170.

-making fusion constructs with the ABC transporter; the specification provides all elements for making such a construct.

-determining if the obtained hybrid protein is capable of functioning as a biosensor; the specification provides all elements for making such a construct; furthermore the herewith attached figures 1 and 2 confirm the pertinence of the selected tests.

Moreover, as previously the arrangement of these elements into functional hybrid proteins is within the skill of the artisan in the field of molecular biology, especially given the number of exemplified hybrid proteins disclosed in the present specification.

Accordingly, the Applicants respectfully request that these grounds of rejection be withdrawn.

Rejection—35 U.S.C. §102

Claims 1, 11, 12, 34 and 35 were rejected under 35 U.S.C. 102(b) as being anticipated by Vankeerberghen et al., Biochem. 38:14988. The Applicants traverse this rejection since Vankeerberghen does not disclose a fusion protein comprising MRP1, a spacer, and Kir 6.2

as required by the present claims as they read on the elected species as well as on a genus of hybrid proteins comprising an ABC transporter and Kir family ion channel element.

Vankeerberghen does not fuse/associate a Kir family ion channel and an ABC transporter. Rather, this document describes a classical approach using chimeric proteins to understand the role of the R domains of CFTR. The R domain is a small cytoplasmic part in the middle of CFTR which links domains NBD1 and TMD1. To investigate the role of this domain, deletions and replacements by small parts (at most 45 residues out of 1300) of equivalent domain in MDR1 are made and the effects of these maneuvers on the function of CFTR (a cAMP-regulated chloride channel) are tested. Vankeerberghen studied CFTR, not MDR1; did not fuse a functional MDR1 to CFTR, or use CFTR as a reporter of MDR1 activity. Rather, Vankeerberghen simply used the CFTR current as a reporter of CFTR function. Accordingly, this rejection may now be withdrawn.

Rejection—35 U.S.C. §102

Claims 1, 21-24, 34 and 35 were rejected under 35 U.S.C. 102(b) as being anticipated by Takano et al., J. Physiol. 512: 395. The Applicants traverse this rejection since Takano does not disclose a fusion protein comprising MRP1, a spacer, and Kir 6.2 as required by the present claims as they read on the elected species (or a genus of ABC transporter + Kir family ion channel hybrid proteins).

Takano describes a chimeric strategy involving fusions between the two isoforms Kir6.1 and Kir6.2 to identify the regions within these channels involved in the nucleotide modulation of gating. This is a classical mutagenic approach widely employed in structure-function studies of ions-channels which involves exchanging homologous regions of Kir 6.1 and Kir 6.2 to see which region is responsible for which feature of each channel. The constructions are termed hybrid proteins because they share parts from two different proteins

but they bear no relation to fusion proteins where the ion channel reports on an attached ABC transporter protein. Accordingly, this rejection may now be withdrawn.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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